



Amendments to Specification

Please amend the Specification as follows:

**Please add the following new paragraphs after line 24 of page 7:**

SEQ ID NO:17 is the nucleotide sequence of the additional bases (GGGAATTCCATATG) in the 5' primer.

SEQ ID NO:18 is the nucleotide sequence of the Not I site and 10 additional bases added to the 3' primer.

SEQ ID NO:19 is the nucleotide sequence of the 12 nucleotide intervening sequence used to create plasmid pML64.

SEQ ID NO:20 is the nucleotide sequence of the first 12 nucleotides of the (*Phaseolus vulgaris*) phaseolin (7S seed storage protein) promoter in plasmid AS3.

**Please replace the following paragraphs:**

**Paragraph at page 35, lines 17-27:**

mRNA was purified from this nucleic acid fraction using the mRNA purification kit from Pharmacia. Approximately 12 µg of mRNA was obtained. Thirteen ng of the polyadenylated mRNA was used as template for amplification from oligo-dT using a GeneAmp® RNA-PCR kit (Perkin Elmer Cetus, part no. N808-0017). The reverse transcriptase reaction was run for 30 min at 42°C. For the PCR amplification, Vent™ DNA polymerase (New England Biolabs) was substituted for the DNA polymerase supplied by the kit manufacturer and an additional 2 µL of 100 mM magnesium sulfate was added to each 100 µL reaction. The 5' primer had the sequence shown in SEQ ID NO:3 and consists of bases 57 to 77 in SEQ ID NO:1 with the additional bases 5'-GGGAATTCCATATG-3' (SEQ ID NO:17) added to encode an Nde I site in the primer with eight additional 5' bases to enhance the restriction enzyme activity against the sequence.

**Paragraph at page 35, line 28-page 36, line 11:**

The 3' primer had the sequence shown in SEQ ID NO:4 and consists of the reverse complement of bases 1566 to 1586 in SEQ ID NO:1 with the additional bases 5'-AAGGAAAAAGCGGCCGC-3' (SEQ ID NO:18) added to provide a Not I site in the primer and ten additional bases to enhance restriction digestion. The PCR reaction was run for 35 cycles at a 52° annealing temperature and 1.5 min extension

time. A product of about 1550 base pairs was obtained and purified by passage through an Amicon 50 microfuge filter followed by extraction with an equal volume of 1:1 phenol:chloroform, extraction of the upper layer of the phenol:chloroform separation with one volume of chloroform and precipitation with ethanol. Five  $\mu$ g of the resulting, clean PCR product was digested overnight at 37° with both Nde I and Not I. The restriction enzyme digest was de-proteinized by the above described phenol:chloroform extraction procedure and ligated into 2  $\mu$ g of pET24aT7 expression vector (Novogen) that had also been digested with Nde I and Not I and treated with calf intestine alkaline phosphatase to hydrolyzed the terminal phosphates. The ligation mixture was used to transform electocompetant DH 10B *E. coli* cells and transformants were selected by growth on plates containing 30 mg l<sup>-1</sup> kanamycin. Eighteen single colonies from the transformation plate were picked and placed in 100  $\mu$ L of sterile water. Forty  $\mu$ L of the cell mix was used as the DNA template in a PCR reaction run with Taq™ polymerase (Perkin Elmer) using the primers and PCR conditions that were initially used to isolate the cDNA insert. Six colonies served as template to amplify a product of the correct size. The remaining 60  $\mu$ L of the cell mix from these clones was grown in overnight culture and plasmid DNA preparations were made from each clone. The purified plasmid from each of the six clones was used to transform electrocompetant DE 3 *E. coli* cells.

**Paragraph at page 39, line 16-page 40, line 11:**

The pML70 vector contains the KTi3 promoter and the KTi3 3' untranslated region and was derived from the commercially available vector pTZ18R (Pharmacia) via the intermediate plasmids pML51, pML55, pML64 and pML65. A 2.4 kb Bst BI/Eco RI fragment of the complete soybean KTi3 gene [Jofuku and Goldberg *supra*], which contains all 2039 nucleotides of the 5' untranslated region and 390 bases of the coding sequence of the KTi3 gene ending at the Eco RI site corresponding to bases 755 to 761 of the sequence described in Jofuku et al., (1989) *Plant Cell* 1:427-435, was ligated into the Acc I/Eco RI sites of pTZ18R to create the plasmid pML51. The plasmid pML51 was cut with Nco I, filled in using Klenow, and religated, to destroy an Nco I site in the middle of the 5' untranslated region of the KTi3 insert, resulting in the plasmid pML55. The plasmid pML55 was partially digested with Xmn I/Eco RI to release a 0.42 kb fragment, corresponding to bases 732 to 755 of

the above cited sequence, which was discarded. A synthetic Xmn I/Eco RI linker containing an Nco I site, was constructed by making a dimer of complementary synthetic oligonucleotides consisting of the coding sequence for an Xmn I site (5'-TCTTCC-3') and an Nco I site (5'-CCATGGG-3') followed directly by part of an Eco RI site (5'-GAAGG-3'). The Xmn I and Nco I/Eco RI sites were linked by a short intervening sequence (5'-ATAGCCCCCAA-3'; SEQ ID NO:19). This synthetic linker was ligated into the Xmn I/Eco RI sites of the 4.94 kb fragment to create the plasmid pML64. The 3' untranslated region of the KTi3 gene was amplified from the sequence described in Jofuku et al., [supra] by standard PCR protocols (Perkin Elmer Cetus, GeneAmp PCR kit) using the primers ML51 and ML52. Primer ML51 contained the 20 nucleotides corresponding to bases 1072 to 1091 of the above cited sequence with the addition of nucleotides corresponding to Eco RV (5'-GATATC-3'), Nco I (5'-CCATGG-3'), Xba I (5'-TCTAGA-3'), Sma I (5'-CCCGGG-3') and Kpn I (5'-GGTACC-3') sites at the 5' end of the primer. Primer ML52 contained the exact complement of the nucleotides corresponding to bases 1242 to 1259 of the above cited sequence with the addition of nucleotides corresponding to Sma I (5'-CCCGGG-3'), Eco RI (5'-GAATTC-3'), Bam HI (5'-GGATCC-3') and Sal I (5'-GTCGAC-3') sites at the 5' end of the primer. The PCR-amplified 3' end of the KTi3 gene was ligated into the Nco I/Eco RI sites of pML64 to create the plasmid pML65. A synthetic multiple cloning site linker was constructed by making a dimer of complementary synthetic oligonucleotides consisting of the coding sequence for Pst I (5'-CTGCA-3'), Sal I (5'-GTCGAC-3'), Bam HI (5'-GGATCC-3') and Pst I (5'-CTGCA-3') sites. The linker was ligated into the Pst I site (directly 5' to the KTi3 promoter region) of pML65 to create the plasmid pML70.

**Paragraph at page 40, lines 12-29:**

The pCW108 vector contains the bean phaseolin promoter and 3' untranslated region and was derived from the commercially available pUC18 plasmid (Gibco-BRL) via plasmids AS3 and pCW104. Plasmid AS3 contains 495 base pairs of the bean (*Phaseolus vulgaris*) phaseolin (7S seed storage protein) promoter starting with 5'-TGGTCTTTGGT-3' (SEQ ID NO:20) followed by the entire 1175 base pairs of the 3' untranslated region of the same gene [see sequence descriptions in Doyle et al., (1986) *J. Biol. Chem.* 261:9228-9238 and Slightom et al., (1983) *Proc. Natl. Acad.*

*Sci. USA* 80:1897-1901; further sequence description may be found in World Patent Publication WO911/3993] cloned into the Hind III site of pUC18. The additional cloning sites of the pUC18 multiple cloning region (Eco RI, Sph I, Pst I and Sal I) were removed by digesting with Eco RI and Sal I, filling in the ends with Klenow and religating to yield the plasmid pCW104. A new multiple cloning site was created between the 495 bp of the 5' phaseolin and the 1175 bp of the 3' phaseolin by inserting a dimer of complementary synthetic oligonucleotides consisting of the coding sequence for a Nco I site (5'-CCATGG-3') followed by three filler bases (5'-TAG-3'), the coding sequence for a Sma I site (5'-CCCGGG-3'), the last three bases of a Kpn I site (5'-TAC-3'), a cytosine and the coding sequence for an Xba I site (5'-TCTAGA-3') to create the plasmid pCW108. This plasmid contains unique Nco I, Sma I, Kpn I and Xba I sites directly behind the phaseolin promoter.